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ANTAGONISTIC EFFECT OF *TRICHODERMA* SPP. PRESENT IN SPENT MUSHROOM SUBSTRATE AGAINST *RHIZOCTONIA SOLANI* CAUSING DAMPING-OFF AND ROOT ROT IN TOMATO

SURESH VERMA, ASHWANI KUMAR, JAGDEEP SINGH,
SURJEET SINGH & R. S. RANA

Department of Plant Pathology, College of Agriculture, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

ABSTRACT

Spent mushroom substrate (SMS) is the result of mushroom houses; remain after the harvest of mushroom crop which having bio-agents and rich in nutrients. It not only supply nutrition to the soil but also helps in management of soil-borne plant pathogens. In present studies the bio-agents Trichoderma spp., named as T. harzianum and T. viride were isolated from SMS and efficacy of bio-agents were tested by seed treatment in screen house as well as SMS application in nursery conditions; against R. solani causing damping-off and root rot in tomato. It was reported that, seed treatment of tomato with T. harzianum along with addition of SMS @ 15% showed maximum (88.33%) seed germination and minimum (11.28%) seedling mortality. Similarly, in nursery conditions, addition of SMS @ 2.5 kg/m^2 was found to be most effective which showed the maximum seed germination (78.70%) and disease control (63.19%); along with low (15.88%) seedling mortality.

KEYWORDS: Spent Mushroom Substrate, Trichoderma, Rhizoctonia Solani and Bio-Agents

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Original Article

INTRODUCTION

The ever-increasing human demand for protein-rich food and the inefficiency of conventional methods have resulted in the need to explore alternatives for low cost production of unconventional protein-rich food (Mukherjee and Nandi, 2004). One of the options is edible fungi or mushrooms belonging to the Basidiomycetes. To date, the mushroom industry is an industry with world production greater than 25 mi tones. The current largest mushroom producer of mushrooms, China has attained more than 20 mi tones and accounted for over 80 per cent of the world's mushroom production (Li, 2012).

Growing of mushrooms on large scale have been providing employment for several people in the developed and developing countries of the world (Aina *et al.*, 2012a). It has been widely reported that commercial mushroom cultivation use substrates like wheat straw, poultry manure, gypsum, horse manure etc. (Jonathan *et al.*, 2012b). Spent Mushroom Substrate (SMS) or Spent Mushroom Compost (SMC) is a by-product of mushroom production. It results from the production growing media that is removed from the mushroom farm after the completion of harvest. Correspondingly, for every kilogram of mushroom cultivated, 3-5 kg of SMS is generated (Lau *et al.*, 2003). Singh *et al.* (2011) stated that average farm discards about 24 tonnes of SMS per month. In India, the annual production of mushroom is 41000 mi tones from which approximately 1,64,000 mi tones of SMS

is produced. In Haryana alone, more than 50000 tonnes of SMS is generated from the mushroom farms (NHB, 2013). In Ireland, approximately 2,54,000 tones of SMS is generated each year (Barry *et al*., 2012) and in Netherlands, more than 8,00,000 tones of SMS is produced per year (Oei and Albert, 2012).

SMS possesses the quality of good organic manure for raising healthy crops of cereals, fruits, vegetables and ornamental plants. In addition to its ability of reclaiming the contaminated soil, SMS is utilized for nutritionally poor soil, neutralizing acidic soil and in other cases for improving polluted sites (Ahlawat *et al*., 2005). Holcomb (2001) found that organic manure including spent mushroom compost encouraged a population of antagonistic microorganisms such as *Trichoderma* spp., *Bacillus* spp. that interfere with the activity of plant pathogens.

Noble and Coventry (2005) reviewed the various combinations of biological control agents (including *T. harzianum*) and organic amendment that were reported to control soil borne plant pathogens. They stated that such combinations could significantly reduce the disease caused by *R. solani*. Rivera *et al.,* (2004) found that organic amendment or compost reduces disease caused by *R. solani* in a variety of crops. SMS has also been shown to suppress various soil fungi and soil borne plant diseases as well as to increase microbial densities in soils (Davis *et al.,* 2005). The presence and antagonism of different fungal microorganisms has been worked out by Viji *et al.,* (2003) and Philippoussis *et al.,* (2004) in which they have reported that the organic substrates such as spent mushroom compost can suppress a variety of plant pathogenic fungi including soil borne pathogens like *Rhizoctonia* spp. and *Pythium* spp.

So, the thousand tons of waste obtained from mushroom cultivation known as SMS, is being dumped on the road-side and awareness about its proper utilization is not much available. In Haryana, being the leading state in cultivation of button mushroom, SMS in excess of 50,000 tones is generated per year which is not properly utilized. So, keeping this in view, the present work was undertaken for evaluation of antagonistic effect of beneficial mycoflora present in SMS against *Rhizoctonia solani* causing damping-off and root rot in tomato.

MATERIALS AND METHODS

The present experiments were carried out at screen house and field of Department of Plant Pathology, CCS Haryana Agricultural University, Hisar during 2013-2014. The details of materials and methods used in present investigation are as follows:

Evaluation of Bio-Agents and Addition of Sms Against *R. Solani*

Isolated fungal bio-agents were evaluated for their efficacy by seed treatments along with addition of SMS in managing *R. solani* in tomato cultivar ‘Hisar Arun’ in screen house. Evaluation of addition of SMS was also studied in nursery conditions on same cultivar.

Evaluation of Fungal Bio-Agents as Seed Treatment Along with Addition of Sms in Screenhouse

The experiment was laid down in pots in completely randomized design to determine the combined effects of seed treatment with antagonists and addition of SMS for the management of *R. solani* in tomato.

Field soil filled in 12” diameter earthern pots was inoculated with *R. solani* inoculum prepared by above said method @ 5 gm/kg soil. The soil was kept moist for three days for the stabilization of the pathogen. After three days, SMS was added in the pots at three different rates i.e. 50, 100 and 150 gm per kg of soil i.e. 5, 10 and 15 per cent respectively. After 3 days of addition of SMS in pots, 15 seeds treated with bio-agents were sown in each pot. Untreated seeds sown in

inoculated soil served as control treatments. Each treatment has three replications. Observations on disease data were recorded upto 30 days. Per cent seed germination was calculated after two weeks and per cent seedling mortality and per cent disease control was calculated after four weeks of sowing by following formulas:

$$\% \text{ Seed germination} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

$$\% \text{ Seedling mortality} = \frac{\text{Seedling stand after two weeks of sowing} - \text{Seedling stand after four weeks of sowing}}{\text{Seedling stand after two weeks of sowing}} \times 100$$

$$\% \text{ Disease control} = \frac{\text{Seedling mortality in control} - \text{Seedling mortality in treatment}}{\text{Seedling mortality in control}} \times 100$$

Evaluation of Addition of SMS in Nursery Beds

This study was conducted on Plant Pathology field using sick plot technique. The experiments were laid down in 1 m^2 nursery beds in randomized block design to determine the effect of addition of SMS for management of *R. solani* in tomato.

Flat nursery beds each of size 1 m^2 and 6 cm height were prepared and fine soil tilth was made. Each nursery bed was inoculated with *R. solani* inoculum @ 150 gm/m^2. The soil was kept moist for three days for the stabilization of the pathogen. After three days, SMS was added at different rates i.e. 1.5, 2 and 2.5 kg per bed and the beds was left as such for 5 days. Untreated seeds of tomato were sown in each bed at the spacing of 20x10 cm. Seeds sown in unadded inoculated nursery beds served as control treatments. Each treatment has three replications. Observations on disease data were recorded upto 30 days. Per cent seed germination was calculated after two weeks; per cent seedling mortality and per cent disease control were calculated after four weeks of sowing.

STATISTICAL ANALYSIS

The experimental data were statistically analyzed by “Analysis of Variance” and the significance of treatment was judged with the help of F-test and t-test.

RESULTS AND DISCUSSIONS

Evaluation of Fungal Bio-Agents as Seed Treatment along with Addition of Sms in Screen House

An evident from Table 1 and Figure 1 that, the seed germination after 15 DAS was significantly increased when seeds were treated with bio-agents, however, *T. harzianum* found to be superior over *T. viride* as these showed significant difference in seed germination. When the seeds were treated with bio-agents without addition of SMS, the highest per cent seed germination was observed in the pots treated with *T. harzianum* (81.66%) followed by *T. viride* (74.99%). Addition of

SMS significantly increased the seed germination of tomato. The results reveal that the maximum per cent seed germination was observed when the SMS was added at the rate of 15 per cent along-with seed treatment with bio-agents. *T. harzianum* showed highest per cent seed germination (88.33%) followed by *T. viride* (84.99%).

Table 1: Efficacy of *Trichoderma* Spp. as Seed Treatment with Addition of SMS on Seed Germination of Tomato Under screen House Conditions

Treatment[#]	% Seed Germination*	% Increase Over Control
ST with *T. harzianum*	81.66 (64.84)	30.61
ST with *T. viride*	74.99 (60.10)	24.44
ST with *T. harzianum* + 5% SMS	83.33 (65.98)	32.00
ST with *T. harzianum* + 10% SMS	86.66 (68.87)	34.61
ST with *T. harzianum* + 15% SMS	88.33 (70.49)	35.25
ST with *T. viride* + 5% SMS	79.99 (63.56)	29.16
ST with *T. viride* + 10% SMS	81.66 (64.69)	30.61
ST with *T. viride* + 15% SMS	84.99 (67.26)	33.33
Control (Untreated inoculated)	56.66 (48.81)	-
C.D. (p=0.05) 5.66		

[#] ST = seed treatment

* Figures in parentheses are angular transformed values

At 5 per cent and 10 per cent addition of SMS, the per cent seed germination in *T. harzianum* and *T. viride* treatments were 83.33 per cent and 79.99 per cent; and 86.66 per cent and 81.66 per cent while that of control treatment was 56.66 per cent.

After four weeks the recorded seedling mortality in SMS unadded treatments is highest as compared to SMS added treatments (Table 2 and Figure 1). The maximum disease control, 75.93 per cent, was found to be in seed treatment of *T. harzianum* added with 15 per cent SMS. Per cent seedling mortality decreased as the rate of SMS was increased. Both, *T. harzianum* and *T. viride* has the significant effect in managing the pathogen when added with SMS @ 10 and 15%. *T. harzianum* showed 18.10, 15.42 and 11.28 per cent seedling moratilty and *T. viride* showed 22.65, 16.34 and 13.61 per cent seedling mortality when the rate of SMS added was 5, 10 and 15 per cent, respectively.

Table 2: Efficacy of *Trichoderma* Spp. as Seed Treatment with Addition of SMS on Seedling Mortality of Tomato under Screen House Conditions

Treatment[#]	% Seedling Mortality*	% Disease Control
ST with *T. harzianum*	24.41 (29.43)	47.91
ST with *T. viride*	26.40 (30.81)	43.67
ST with *T. harzianum* + 5% SMS	18.10 (25.05)	61.38
ST with *T. harzianum* + 10% SMS	15.42 (23.10)	67.10
ST with *T. harzianum* + 15% SMS	11.28 (19.38)	75.93
ST with *T. viride* + 5% SMS	22.65 (28.24)	51.67
ST with *T. viride* + 10% SMS	16.34 (23.83)	65.13
ST with *T. viride* + 15% SMS	13.61 (21.15)	70.96
Control (Untreated inoculated)	46.87 (43.17)	-
C.D. (p=0.05) 5.62		

[#] ST = seed treatment

* Figures in parentheses are angular transformed values

The potentiality of *Trichoderma* spp. as biocontrol agents of phyto-pathogenic fungi in several crops is well known especially to *Fusarium* spp. and *Rhizoctonia* spp. (Poddar *et al.*, 2004 and Rojo *et al.*, 2007). The antagonistic

activity of *Trichoderma* spp. could be related to their ability to act as biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism (Cook, 2000).

The seed treatment with *Trichoderma* spp. in the present study and the addition of SMS along with effectively controlled the disease in tomato caused by *R. solani*. The ability of *Trichoderma* to reduce diseases caused by soil borne pathogens is well known and it is related to the antagonistic properties of *Trichoderma*, which involve parasitism and lysis of pathogenic fungi and/or competition for limiting factors in the rhizosphere mainly iron and carbon (Sivan & Chet, 1986). Another mechanism has been suggested by Kleifeld and Chet (1992) related to *Trichoderma*-induced resistance in host plants to fungal attack. *T. viride* and *T. harzianum* were reported by several workers as the best antagonists against several soil and seed borne plant pathogens (Poddar *et al.*, 2004).

Jayaraj *et al.* (2006) reported that the seed treatment with *T. harzianum* formulations reduced the incidence of damping-off disease of tomato by up to 74 per cent and enhanced plant biomass under greenhouse and field (Tamil Nadu, India) conditions.

Evaluation of Addition of Sms in Nursery Beds

SMS was added to the flat nursery beds of 1x1 m^2 size which were inoculated with *R. solani* infested wheat grains. Tomato seeds were sown and per cent seed germination and per cent seedling mortality were calculated.

The results presented in Table (3&4) and Figure 2 show that addition of SMS @ 2.5 kg/m^2 showed maximum per cent seed germination (78.70%) and minimum per cent seedling mortality (15.88%). The minimum per cent seed germination (71.29%) and maximum per cent seedling mortality (27.64%) was observed when the rate of SMS added was 1.5 kg/m^2 while 76.84 per cent seed germination and 19.78 per cent seedling mortality was obtained from the beds where SMS was added @ 2 kg/m^2 while the control beds showed 51.84 per cent seed germination and 43.15 per cent seedling mortality where no SMS was added.

In nursery conditions, SMS showed a significant effect on the seed germination and seedling mortality when added @ 2.0 and 2.5 kg/m^2. The maximum seed germination (78.70%) was resulted when 2.5 kg was added in 1 m^2 area. The increase in seed germination over the un-added control was 27.28 per cent @ 1.5 kg which increased to 32.53 per cent @ 2.0 kg and to 34.12 per cent @ 2.5 kg. Similar trend was followed at 30 DAS where the per cent seedling mortality was found to be decreased from 43.15 to 27.64 per cent, 19.78 per cent and 15.88 per cent with the increase in rate of SMS from 0 kg to 1.5 kg, 2.0 kg and 2.5 kg respectively. The maximum disease control (63.19%) was in the beds added with 2.5 kg SMS which was significantly higher than the beds aided with 1.5 kg (35.94%).

Table 3: Effect of Addition of SMS on Seed Germination of Tomato in Nursery beds Inoculated with *Rhizoctonia solani*

SMS Added (kg/m^2)#	% Seed Germination*	% Increase Over Control
1.5 kg	71.29 (57.65)	27.28
2.0 kg	76.84 (61.27)	32.53
2.5 kg	78.70 (62.56)	34.12
Control (Untreated inoculated)	51.84 (46.04)	-
C.D. (p=0.05) 5.65		

size of bed = 1x1 m

* Figures in parentheses are angular transformed values

Table 4: Effect of Addition of SMS on Seedling Mortality of Tomato in Nursery Beds Inoculated with *Rhizoctonia solani*

SMS Added (kg/m^2)#	% Seedling Mortality*	% Disease Control
1.5 kg	27.64 (31.67)	35.94
2.0 kg	19.78 (26.39)	54.15
2.5 kg	15.88 (23.34)	63.19
Control (Untreated inoculated)	43.15 (41.00)	-
C.D. (p=0.05) 7.58		

size of bed = 1x1 m

* Figures in parentheses are angular transformed values

Organic soil amendment is another important option and eco-friendly approach for controlling damping off disease by developing suppressive nature of soil (Dey, 2005; Islam *et al.*, 2007). The severity of bean root rot caused by *R. solani* was reduced by compost (Ferrara *et al*., 1996). Several authors studying a variety of crops have found that organic amendment or compost reduces disease caused by *R. solani* (Alabouvette *et al*., 2004; Rivera *et al*., 2004; Noble and Coventry, 2005).

SUMMARY AND CONCLUSIONS

The seed treatment of *Trichoderma* spp. along with addition of SMS had significantly reduced the effect of fungus *R. solani.* SMS added and treated seeds had higher seed germination in comparison to control treatments. For instance, addition of 10 per cent and 15 per cent of SMS had 86.66 per cent and 88.33 per cent germination for seeds treated with *T. harzianum*, 81.66 per cent and 84.99 per cent germination for seeds treated with *T. viride*, respectively. Without addition of SMS *T. harzianum* treated seeds had 81.66 per cent germination while *T. viride* treated seeds had only 74.99 per cent germination. Similarly, seedling mortality was significantly reduced by addition of SMS along with seed treatment with bio-agents. The disease control was enhanced by 67.10 per cent and 75.93 per cent with seed treatment of *T. harzianum* added with 10 per cent and 15 per cent SMS respectively. For seeds treated with *T. viride* and added with 10 per cent and 15 per cent SMS showed 67.10 per cent and 75.93 per cent disease control.

On the other hand, under nursery conditions, the amendment of SMS had a significant increase in seed germination in comparison to un-added beds. The maximum seed germination was in the beds amended with 2.5 kg SMS (78.70%). The beds aided with 1.5 kg and 2.0 kg of SMS showed 71.29 per cent and 76.84 per cent seed germination respectively while control un-added beds had seed germination of 51.84 per cent. Similarly after four weeks of sowing, the disease control was maximum (63.19%) in the beds aided with 2.5 kg SMS followed by the beds aided with 2 kg (54.15%) and 1.5 kg (35.94%).

PLATE - 1

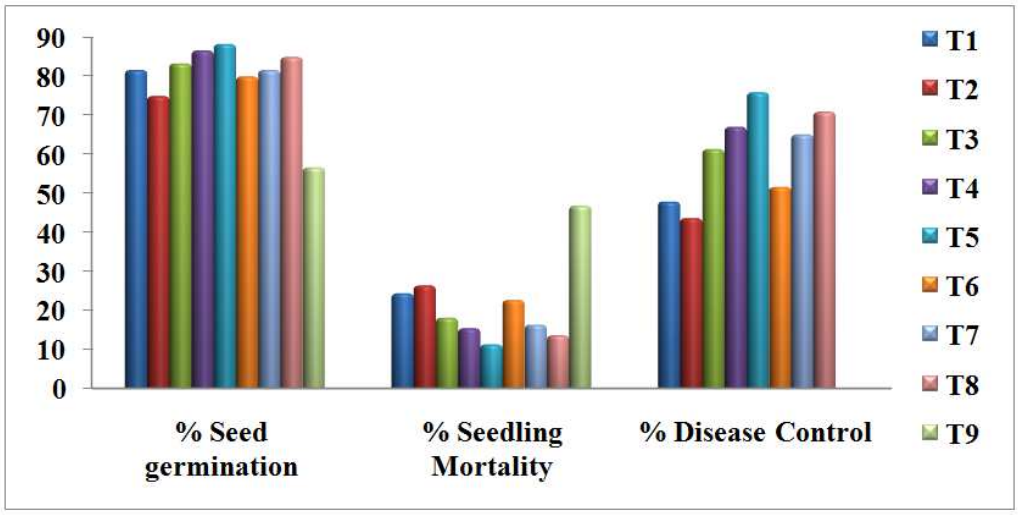


Figure 1: Effect of antagonists as seed treatment with addition of SMS on Tomato Against*r. solani* under screen house conditions

T1 = ST with *T. harzianum;* T2 = ST with *T. viride;* T3 = ST with *T. harzianum* + 5% SMS; T4= ST with *T. harzianum* + 10% SMS; T5 = ST with *T. harzianum* + 15% SMS; T6 = ST with *T. viride* + 5% SMS; T7 = ST with *T. viride* + 10% SMS; T8 = ST with *T. viride* + 15% SMS; T9 = Control

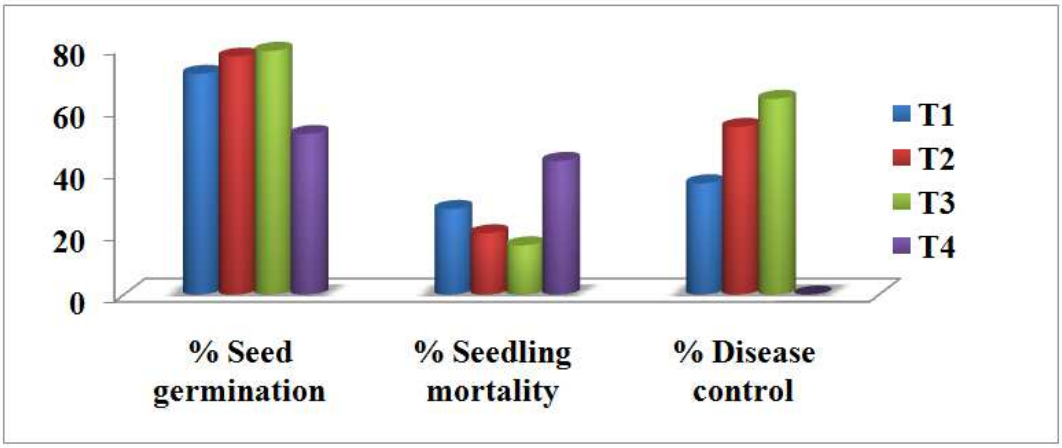


Figure 2: Effect of Addition of Spent Mushroom Substrate on Tomato in Nursery Beds Inoculated with *Rhizoctonia solani*

T1= 1.5 kg SMS/m^2; T2= 2.0 kg SMS/m^2; T3= 2.5 kg SMS/m^2; T4= control

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